RELATIONSHIP BETWEEN CYTOKINE INTERLEUKIN 10 WITH DEGREES OF HIRSCHSPRUNG-ASSOCIATED ENTEROCOLITIS (HAEC) BASED ON TEITELBAUM CRITERIA

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ABSTRACT:

Introduction. Hirschsprung's disease is a developmental disorder of the intrinsic component of the enteric nervous system characterized by the absence of ganglion cells in the myenteric plexus and submucosa in the distal intestine, due to failure of cephalocaudal migration of ganglion cells at 12 weeks gestation, which causes aganglionic conditions in part or the whole of the colon. Hirschsprung's Associated Enterocolitis (HAEC) is a cause of morbidity and mortality in Hirschsprung patients. The reported incidence of HAEC varies widely, ranging from 6 to 60% definitive pre-operatively and from 25 to 37% postoperatively. Among all researches have been published in international and national journals, there was no study explore the relationship between IL-10 and HAEC degrees. Based on this, the researcher is interested in conducting further studies on the role of IL-10 in HAEC because it has almost similar mechanisms where there is damage to the colon wall.

Methods. Children suffering from Hirschsprung's disease without HAEC and Hirschsprung’s disease with HAEC were examined for the degree of HAEC based on colon histopathology and IL-10 level according to Teitelbaum's criteria. Analysis is performed using computer-based software and determined by regression analysis.

Results. the majority hirschprung sample was male, where 11 patients (73.4%) of hirschprung were male compared to female which only 4 patients (36.6%) from a total of 15 patients we studied, most frequent HAEC grades were grade 3,4 and grade 5 (59.9%), analysis IL 10 in Hirschsprung without HAEC and Hirschsprung accompanied by HAEC using the independent t-test (p = 0.007) with correlation showed r = -0.774 using Pearson test.

Conclusion. There is a very strong positive relationship / correlation between histopathological grade and IL10 levels, the higher the histopathological grade, the lower the IL10 levels, on the contrary the lower the grade histopathology, the higher the IL10 level.

Keywords: Hirschsprung-Associated Enterocolitis (HAEC), Pearson Test, Crohn's Disease.

I. INTRODUCTION

Hirschsprung's disease is a developmental disorder of the intrinsic component of the enteric nervous system characterized by the absence of ganglion cells in the myenteric plexus and submucosa in the distal intestine, due to failure of cephalocaudal migration of ganglion cells at 12 weeks gestation, which causes aganglionic conditions in part or the whole of the colon. (J Kessmann et al, 2006).
Hirschsprung's Associated Enterocolitis (HAEC) is a cause of morbidity and mortality in Hirschsprung patients. The incidence of HAEC worldwide ranges from 6-58%, while the mortality rate in HAEC is quite high, namely between 6-30. (J Kessmann et al., 2006) The reported incidence of HAEC varies widely, ranging from 6 to 60% definitive pre-operatively and from 25 to 37% postoperatively (Gosain et al., 2017).

The etiology and pathophysiology of HAEC are poorly understood, resulting in a broad and varied clinical entity ranging from abdominal distension to septic shock with multiorgan failure. (Dore et al., 2018).

When viewed from the histological point of view, HAEC is characterized by the presence of cryptitis, with extensive inflammation and a neutrophilic infiltration of the crypts. In the mild form of HAEC, its contains crypto and retains mucin as occurs in cystic fibrosis. More advanced form of HAEC, there is a microscopic progression of the disease, which is characterized by crypt abscesses, intraluminal accumulation of fibrinopurulent debris, mucosal ulceration leading to transmural necrosis and intestinal perforation. These histopathological changes have been classified into 5 grades. According to the grading system proposed by Teitelbaum, this description has been found not only in the aganglionic colonic segment, but also in the normal segment. (Elhalaby et al., 1995; Gosain and Brinkman et al., 2015).

Macrophages have ability to secrete several cytokines in response to pathogens, including Interleukin 1 (IL-1), Interleukin-6 (IL-6), Inteuleukin 12 (IL-12), Tumor Necrosis Factor-α (TNF-α), Chemokine IL -8 and Interleukins 10. Cytokines are small proteins (~ 25 kDa) released by various cells in response to an activation and as response to specific stimuli. These cytokines can act via the autocrine pathway so they could affect the environment in the cells that release them, or via the paracrine pathway by affecting other cells around them. Some cytokines can also act through the endocrine pathway, affecting the surrounding cell environment although their ability depends on the time they enter the circulation and their half-life. (Shankar et al., 2013).

Among all researches have been published in international and national journals, there was no study explore the relationship between IL-10 and HAEC degrees, although there are 3 studies that are closest to the relationship between IL-10 and its relationship with inflammation in the first colon. Guelph University of Ontario Canada in 2015, did a study regrading IL-10 levels in colon of pigs suffering from chronic inflammation and ulcerative inflammation by administering dextran sodium sulfate with the result of decreased IL-10 levels in severe chronic ulcers. (Lackeyram et al., 2017). The second study at the Academic Medical Center Amsterdam is the prevention of colitis using IL-10. (van Montfrans et al., 2002) The third study was more advanced because they investigated the possibility of administering recombinant human IL-10 to inflammatory bowel disease. According to Fedora et al., 2000 that in 2000 at the University of Alberta Canada an injection of Human recombinant IL-10 under the brand name "Tenovil" was injected subcutaneously in patients with mild to moderate Crohn's disease.

While research in Indonesia on IL-10 is still rare, researchers found that studies were conducted by comparing IL-10 levels based on the severity of acne vulgaris. (Rahmayani et al., 2019) With extensive research on IL-10 in Crohn's disease and inflammatory bowel disease, it is proven that this cytokine is a very important marker in diseases related to the digestive tract, moreover Human IL-10 injection has been found for digestive tract treatment.

Based on this, the researcher is interested in conducting further studies on the role of IL-10 in HAEC because it has almost similar mechanisms where there is damage to the colon wall. In addition, anti-inflammatory cytokines work in the opposite way to pro-inflammatory cytokines. It should be noted that IL-10 levels in Hirschsprung disease are compared with Hirschsprung disease patients who have HAEC to assess the possibility of a relationship between HAEC and Interleukin 10 levels.

II. MATERIALS AND METHOD

Subjects

Children suffering from Hirschsprung's disease without HAEC and Hirschsprung’s disease with HAEC were examined for the degree of HAEC based on colon histopathology according to Teitelbaum's criteria.

Procedure

This research was carried out in several steps as follows; recording the identities of patients who meet the inclusion criteria and providing a complete explanation of the purpose and benefits of the study, guardians filling in and signing the informed consent as a sign of consent, extraction 3 cc of venous blood samples in a tube without
anticoagulant to samples for IL-10 levels, blood samples without coagulants were centrifuged and then serum was separated. Serum samples that have been taken will be stored in a refrigerator at -20 C to prevent damage to the sample. IL-10 is tested with HUMAN INTERLEUKIN 10 ELISA Kit produced by Bioassay Technology Laboratory, patient samples are taken from the distal stoma and rectum colon segments in the rectal biopsy procedure. Method of taking histopathological samples: samples that are suspected of being abnormal / aganglionic are taken at 2 cm from the anal patch with a minimum size of 1cm x 1cm and put in a special container filled with buffered formaldehyde to further check the histopathology.

The biopsy sample was taken by a surgeon, a 2cm rectal sample was taken from an analyzer that was suspected of being abnormal with a minimum size of 1cm x 1cm. Then the operator put it in a special container filled with buffered formaldehyde, then sample sent to the anatomical pathology laboratory for evaluation of histopathological grade examinations based on Teitelbaum's criteria.

In the implementation of this research, every action was carried out with the consent and knowledge of the patient who was a research participant through an informed consent and it was declared that they met the ethical requirements to be carried out by the Health Research Ethics Commission (KEPK) Faculty of Medicine, Hasanuddin University - RSPTN UH - Dr. Wahidin Sudirohusodo Makassar.

Methods

Analysis is performed using computer-based software and determined by regression analysis. All data obtained were grouped according to the purpose and type of data, then analyzed using SPSS software as follows:

1. Data regarding the mean IL-10 and Ig G levels of each patient will be measured using standard mean ± SD analysis.
2. Multivariate analysis will be tested using the appropriate statistical test. Hypothesis test results will be presented in the form of tables, diagrams and narratives. The test results were stated as insignificant if the p value was> 0.05, significant if p ≤ 0.05, and very significant, if p <0.01.

III. RESULTS

There were 15 patients with colonic tissue samples from pediatric patients who underwent leveling colostomy or rectal biopsy surgery at our institution, taken for histopathological examination. Specifically, the processed sample is tissue distal to the ganglionic zone of the patient's colon. The histopathological description of HAEC was based on the histopathological diagnostic of Teitelbaum colon then followed by the IL 10 examination in the patient's blood sample. The majority of our sample was male (73%) and the median age for surgery was 3 years with a fairly wide age range from infants aged a few months to 15 years. From our entire study population, 10 samples were taken at the time of the rectal biopsy and the rest when leveling the colostomy was performed. There are 2 samples (13.3%) which are grade 0 or what we classify as normal Hirschsprung without HAEC, 2 samples (13.3%) are grade I, 2 samples (13.3%) are grade III, 4 samples (26.6%) are grade IV, 3 samples (20%) are grade V based on the Teitelbaum classification.
Figure 1. Photo of HAEC histopathological grading sample based on Teitelbaum classification. Grade 0 indicates the absence of ganglion accompanied by hypertrophy of nerve cells in the plexus sub mucosa and sub muscularis, thus indicating Hirschsprung disease without HAEC, while grade I shows widening of crypt and mucin retention, grade II is characterized by cryptitis or two cryptic abscesses. Grade III is characterized by the presence of a crypt abscess, grade IV is characterized by fibrinopurulent debris and mucosal ulceration, grade V is characterized by transluminal necrosis or perforation.

When comparing the relationship between IL-10 levels and colonic histopathological grade, it was found that grade 0 or Hirschsprung without HAEC in 2 people with IL-10 values being 588.52 ± 54.43 pg. / ml. Then in grade 1 the IL-10 value was 522.97 ± 12.38. In grade III the IL-10 value was 255.45 ± 19.55 followed by grade IV, the IL-10 value was 158.15 ± 27.86 and the last grade V obtained IL-10 values of 57.05 ± 30.14. While the total mean of the overall IL-10 value was 211.63 pg. / ml with an SD of 194.49.

There is a significant difference between the IL 10 value in Hirschsprung without HAEC and Hirschsprung with HAEC. We examined whether there is a relationship between IL 10 in Hirschsprung without HAEC and Hirschsprung accompanied by HAEC by using the independent t-test. From the independent value of the T test, the significant value is 0.007 where the value is 0.007 <0.05, concluded that there is a significant difference between the IL 10 value in Hirschsprung disease without HAEC and Hirschsprung disease with HAEC. Correlation coefficients to assess the existence of an association between changes in histopathological grade and IL 10 were calculated using the Pearson test. From the data analysis of this study, it was found that a very strong negative correlation (r = -0.774) means that the IL 10 value is inversely proportional to the colonic histopathological grading where the higher the IL 10 value, the lower the colonic histopathological grading, conversely the lower the IL 10 value, the higher the grading. Colon histopathology and correlation were statistically significant with p = 0.001 (<0.05).

Figure 2. Comparison of mean IL 10 levels based on colonic histopathological grade Discussion.

From our study, it was found that the majority hirschprung sample was male, where 11 patients (73.4%) of hirschprung were male compared to female which only 4 patients (36.6%) from a total of 15 patients we studied. This is in line with a study conducted by (Frykman et al. 2013) of 116 Hirschprung Associated Enterocolitis (HAEC) patients, 99 patients (85.34%) were male while 17 were female (14.66%), while from the previous literature it was said that the average incidence of hirschprung based on the sex ratio is 4: 1, with male predominant. This may occur because on the Y chromosome found in males there is a mutation of the Y-box gene (SOX10) as a sex determinant expressed on the nerve crest which contributes to the peripheral nervous system during embryogenesis. Mutations in the gene (SOX10) have been identified as the cause of megacolon in mice and Waardenberg-Shah syndrome in humans, both of which include abnormalities in the enteric nervous system and pigmentation. (“Grosfeld Pediatric Surgery 6th Edition.pdf,” n.d., p. 1536).

In this study it was also found that the most frequently found HAEC grades were grade 3,4 and grade 5 which were an advanced stage where the combination of the three alone had reached (59.9%) this is in accordance with the research conducted by Teitelbaum that most HAEC was found advanced stage where 88% of patients with HAEC are already in grade 3 or above. (“Grosfeld Pediatric Surgery 6th Edition.) Whereas comparing the same research conducted by (Mariana et al., 2020) and (Sunggiardi et al., 2020) also patients with HAEC who have been examined

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for colonic histopathology grading in Makassar, it is found that the results are more or less the same that the average patient those who seek treatment have arrived at an advanced stage. This may be because our hospital is a tertiary referral hospital for Eastern Indonesia. So that most cases are referrals patients who come at an advanced stage.

In this study, it was found that the levels of IL 10 in each group were as follows: in Hirschsprung disease without HAEC was found in 2 people with an IL value of 550.09 ± 627.01 pg/ml. Then in grade I, the IL 10 value was 514.2 ± 531.73. In grade II, the IL 10 value was 270.69 ± 301.28, in grade III the IL 10 value was 211.63 ± 239.28. followed by grade IV, the IL 10 value was 133.26 ± 192.49. and the last, in grade V, the IL 10 value was 24.77 ± 84.46. It is found that IL 10 levels in Hirschsprung Disease without HAEC are higher than those in Hirschsprung with HAEC as a comorbid, where IL 10 levels are decreasing over time along with the increasing histopathological grading of HAEC based on Teitelbaum criteria which indicates disease progression. Where the value is inversely proportional to the IL 10 value, this is in accordance with the initial aim of this study to assess the comparison between IL 10 levels in Hirschsprung disease without HAEC compared with Hirschsprung disease experiencing HAEC based on Teitelbaum criteria and assessing whether IL 10 can be an indicator of disease progression in Hirschsprung Associated Enterocolitis (HAEC).

The average level of IL 10 in this study was 211.63 pg / ml with an SD of 194.49. There were 13 patients (86.6%) suffering from HAEC whereas only 2 patients (14.4%) were hirschsprung patients without HAEC. Obtained a very strong positive correlation (r = 0.774) and had a statistically significant relationship p = 0.001 (<0.05). The lower the IL 10 level, the higher the degree of HAEC. This is in accordance with the hypothesis of this study that there is a relationship between colonic histopathological grade in children with Hirschprung disease who has HAEC and levels of the cytokine IL 10.

In patients with hirschshprung disease due to aganglionic in the colon, mechanical obstruction occurs so that there is a buildup of feces which results in bacterial overgrowth, deficiency of sucrase, changes in colonic mucin. Decreased GALT defense. This results in an imbalance of the microbiome. Coupled with the widening of the permeability of the intestinal wall which makes it easier for bacteria to adhere to the unprotected intestinal epithelium resulting in inflammation of the colon wall. Infection of the bacterial wall will trigger dendritic and mast cells to release pro-inflammatory cytokines which function to eliminate bacteria. With the release of pro-inflammatory cytokines, Th Helper cells release anti-inflammatory cytokines such as IL 10 to suppress the excess amount of pro-inflammatory cytokines. Excessive pro-inflammatory cytokines, although useful for eliminating bacteria, have an excessive amount of influence on the damage to the colon wall which leads to ulcerative colitis or HAEC. Increased pro-inflammatory cytokines also suppress the number of anti-inflammatory cytokines less.

The low level of IL 10 in this study which is an anti-inflammatory cytokine which is an antagonist of pro-inflammatory cytokines such as IL 23 indicates that Hirschsprung's Disease Associated Enterocolitis is an autoimmune disease (Mariana et al., 2020)

Functional colon obstruction caused by genetic disorders in which the absence of ganglion cells in the Meissner and Auerbach plexus in Hirschsprung patients can result in bacterial overgrowth. Infection of the colon tissue can trigger a local immune response that can increase the production of co-stimulators and cytokines by Antigen Presenting Cells (APCs). APC will stimulate T cells which will release proinflammatory cytokines such as IL 1.L16.1L23. TNF α simultaneously promotes Th17 cells which will trigger an autoimmune response. And it makes T reg cells release anti-inflammatory mediators such as IL 10 to limit the excessive immune response. (Yeshi et al., 2020)

The immune system that attacks the lining of the digestive intestine can cause chronic inflammation of the digestive tract, repeated inflammation of the digestive tract can result in damage to the intestinal mucosal immune system, immature digestive tract protection and dysbiosis of microorganisms in the intestine. This can cause diarrhea, bloody and foul-smelling bowel movements, abdominal pain, fever, weight loss and even shock. (Mariana et al., 2020)

The low IL 10 is very influential in the occurrence of colitis. Experiments on mice induced with CD45RB high which made mice suffer from colitis compared to other mice that were also induced with CD45RBhigh but with IL 10 was proven to prevent colitis. IL-10 is a regulatory cytokine that inhibits the activation and effector function of T cells, monocytes, and dendritic cells and has an important role as a central regulation in the immune response of the gut, namely IL 10 limits and ultimately stops the inflammatory response. Therefore IL-10 has been considered
an attractive candidate for the treatment of Crohn’s disease. Recent studies have shown systemic administration of rIL-10 in Crohn’s disease is relatively safe and well tolerated. (Van Montfrans et al., 2002)

IV. CONCLUSION

Based on the results of the research and the discussion above regarding the relationship between histopathological grade and IL10 scores, the following conclusions can be drawn: There is a very strong positive relationship / correlation between histopathological grade and IL10 levels, the higher the histopathological grade, the lower the IL10 levels, on the contrary the lower the grade histopathology, the higher the IL10 level.

REFERENCES